HISTOLOGY OF THE CORPORA ALLATA OF MELANOPLUS DIFFERENTIALIS (ORTHOPTERA: SALTATORIA)

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The corpora allata of insects are known to secrete a hormone called "juvenile" or "inhibitory" hormone which is responsible for maintaining the insect tissues in a juvenile condition and therefore retards metamorphosis (Wigglesworth, 1934, 1936, 1940; reviews by Hanström, 1939; Scharrer, 1941, 1948; Bodenstein, 1942; Piepho, 1943; Joly, 1945a; Mendes, 1947). They also play a role in egg production and in the development or function of male and female accessory glands (Wigglesworth, 1936; see reviews). Whether the different physiological effects of the secretion of the corpora allata are brought about by one or more than one hormone is still a controversial matter. The grasshopper *Melanoplus differentialis* has been the subject of experimental study by Pfeiffer (1939, 1945, 1945a) who has shown that hormone from the corpora allata retards metamorphosis and in adult females controls certain metabolic phenomena, the production of oviducal secretion and the deposition of yolk. It was therefore chosen for study of the histology of the corpora allata at different periods of the life history in an attempt to correlate the histological aspects with the experimental data.

Material and Methods

Melanoplus differentialis passes through six nymphal stages before metamorphosis. Histological preparations were made of the corpora allata of male and female nymphs which were killed at daily intervals during the fifth and sixth stages to compare the changes in these glands during a stage that precedes the production of nymphal characters at molting with those occurring prior to metamorphosis, and to determine whether there is any sexual dimorphism in the nymphal glands. Corpora allata from adult males and females killed at significant ages were prepared for comparison of these glands in the two sexes, for comparison with nymphal glands and for study of the histological changes in adult female corpora allata in relation to egg production.

The grasshoppers used in this study were reared as described by Pfeiffer (1945a). The corpora allata were dissected from freshly cut heads submerged in insect saline. For control purposes some of the corpora allata were dissected from heads not immersed in saline; they showed no difference from those dissected when submerged. The ages of the dissected grasshoppers were recorded in terms of the number of days that had elapsed since the preceding molt. The approximate length of the intermolt periods was learned from control nymphs of the same

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molting dates. When the corpora allata were dissected from adult females, the presence or absence of oviducal secretion and yolk was noted, the oocytes nearest the oviducts were measured, and the blood color was recorded. Several fixatives were tried. Of these, Susa's technique gave the best results and therefore was used almost exclusively. The glands were imbedded in paraffin. The sections, seven to eight micra in thickness, were stained with hematoxylin and eosin, tolnidin blue and erythrosin, methylene blue and erythrosin, Masson's trichrome stain (hemalum, erythrosin and safranin) and Foot's modification of Masson's stain (Weigert's hematoxylin, Ponceau de xylidine, acid fuchsin and light green).

I owe to Dr. I. W. Pfeiffer the privilege of studying iron hematoxylin slides which were useful for the counting of chromosomes.

Topography and Morphology

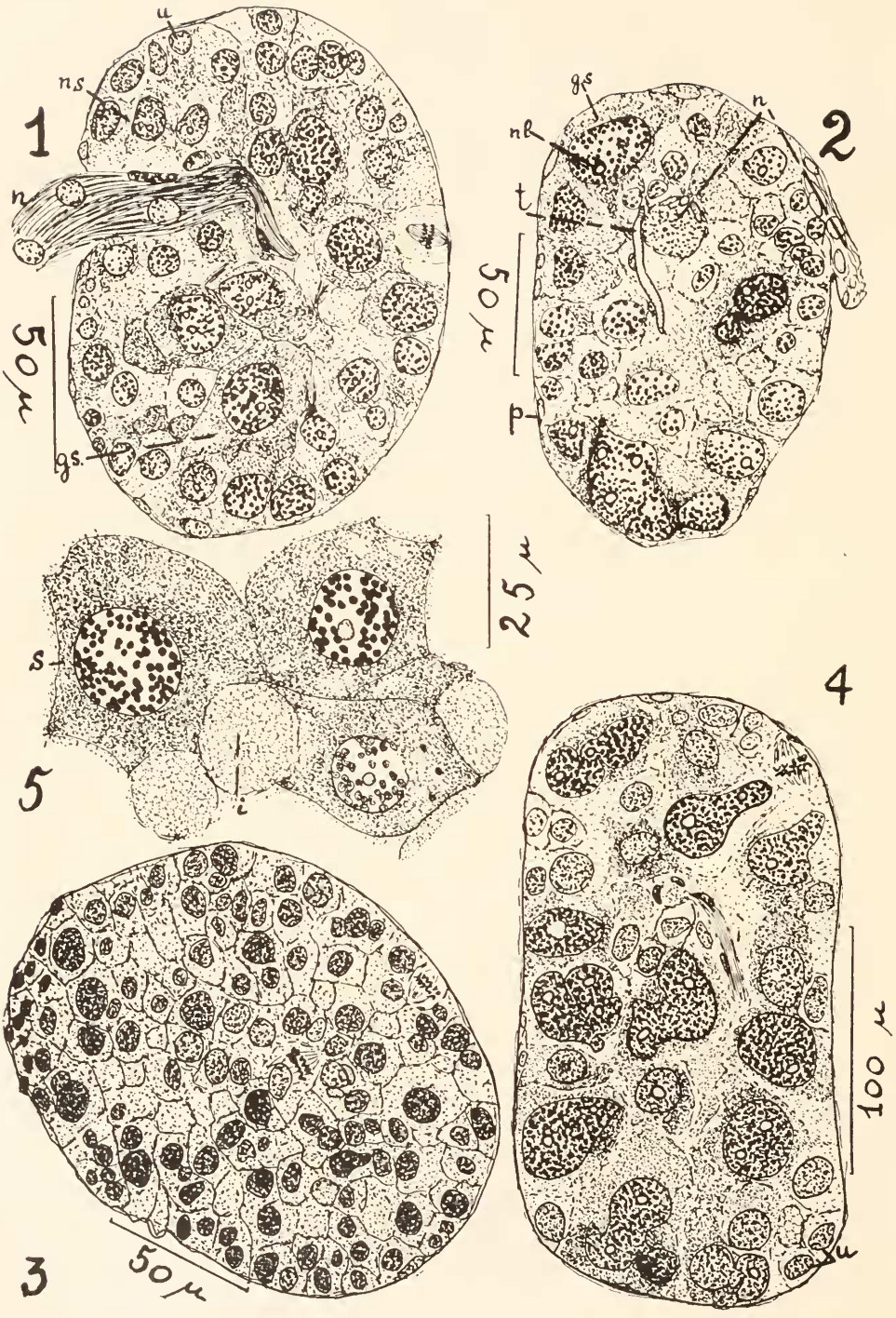
In *Melanoplus* the corpora allata are paired subspherical structures located ventro-laterally to the pharynx, just in front of the crop. The aorta has its anterior opening at about the same level. Therefore, the glands are suspended in a blood lacuna into which the contents of the dorsal vessel are discharged. They are innervated by the nervi corporis allati. There are no nerves emerging from the corpora allata. The nervus corporis allati enters through a slightly depressed point (the hilum, Fig. 1) and ramifies throughout the gland, branches reaching almost all of the glandular cells. Covering the gland is a delicate connective tissue sheath which penetrates the glandular tissue at the hilum and is in direct continuation with the perineurium that covers the nervus corporis allati. The nerve contains a few small cells with spherical nuclei different from the flattened perineurium cells; both kinds are found inside the gland. A trachea penetrates the organ with the nerve, and tracheal cells enter with it. This trachea branches abundantly, so that numerous tracheoles are to be found among the cells. Many fine tracheae may also be seen along the surface of the gland at points away from the hilum. They vary in number with the age of the animal, tracheae being more numerous on the surfaces of old or hypertrophied glands.

Histology

General.—The structure of the corpora allata is fundamentally the same in males, females, nymphs and adults. However, sexual differences and developmental stage and age variations are sufficiently well defined that sex and approximate age of the animal can be estimated from the histological aspect of the corpora allata. Four types of cells may be recognized in the corpora allata: (1) connective tissue cells (of the outer sheath and the perineurium) and tracheal cells; (2) undifferentiated cells; (3) normal secretory cells; and (4) giant secretory or polyploid cells.

The undifferentiated cells (Figs. 1, 4 and 8) are small spherical or polyhedric cells, about 10 to 14 micra in diameter, with relatively large spherical nuclei. These cells have only a small amount of cytoplasm and do not contain secretory granules or vacuoles. They divide mitotically, exhibiting the greatest mitotic activity at the beginning of each developmental stage. However, they may occasionally be found in mitosis at other times during the nymphal or adult stages. Undifferentiated cells are present in the gland at all times.

PLATE I



The normal secretory cells (Fig. 1) may be distinguished from the undifferentiated cells only during active secretory stages of the gland. They vary in volume in accordance with the phase of the cycle of activity of the individual cells. They may become strongly polymorphic, at which time the nucleus is large, usually more or less lobulated, with abundant deeply staining chromatin; one nucleolus is always present. Under the conditions of fixation employed, the cytoplasm of the normal secretory cells is dense and acidophil. During active phases it has a granular appearance due to the presence of strongly acidophil granules. The granules appear first around the nucleus and gradually increase in quantity. When they have become numerous, intracellular vacuoles appear (Fig. 9). These vacuoles vary in size. They may become very large, occupying nearly the entire space at one side of the nucleus. In cells located near the periphery of the gland, the vacuoles are frequently situated on the side of the nucleus toward the center of the gland, under which conditions the cytoplasm in the outer portions of the cell may contain closely crowded acidophil granules. In some cases the content of the vacuoles appears as evenly dispersed granules. In others, particularly the larger ones, it has coagulated into a network. The staining reaction of the vacuolar contents is faintly acidophilic.

When intracellular vacuoles are numerous, vacuoles that are similar in appearance may also be seen between the cells (Figs. 5 and 8), suggesting that secretory material has passed out of the cells into intercellular spaces. At this time, also, particularly when the appearance of the gland suggests the most intense secretory activity, numerous fine lines which suggest "lines of flow" may be seen following somewhat radial courses from central regions of the gland to the periphery. They are especially noticeable in the outer portions of the gland (Fig. 9). When viewed with changing focus, they frequently appear to be between the cells since they are seen most distinctly before the nuclei and cytoplasm come into clear view. At the level of the nucleus the cytoplasm has the usual aspect of evenly

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Explanation of Figures

1. Corpus allatum of fifth instar female nymph killed within 24 hours after molting. The four types of cells are recognizable. Acidophil granules are present in the secretory cells. No intra- or intercellular vacuoles have yet been formed. Mitoses are present. *gs*, giant secretory cell; *n*, nerve entering at the hilum; *ns*, normal secretory cell; *u*, undifferentiated cell.

2. Corpus allatum of fifth instar male nymph killed 8 days after molting. The secretory cells are beginning to decrease in size. Acidophil granules are scarce. A few intercellular vacuoles are still present. The giant secretory cells have polymorphic nuclei. They are more numerous in proportion to the number of normal secretory cells than in female corpora allata (compare with Fig. 1; see also Fig. 4). *gs*, giant secretory cell; *n*, nerve; *nl*, nucleolus; *p*, peripheral connective tissue cell; *t*, trachea.

3. Corpus allatum of sixth instar male nymph killed 2 days after molting. The different types of cells are hardly distinguishable. The cell membranes are distinct. Mitoses are numerous. No secretory material is present.

4. Corpus allatum of sixth instar male nymph killed 4 days after molting. Giant secretory cells with polymorphic nuclei are numerous. Acidophil granules are present near the nuclei. No intercellular vacuoles have as yet been formed. Occasional mitoses are still present. *u*, undifferentiated cells.

5. Three normal secretory cells from a corpus allatum of a sixth instar female nymph killed five days after molting. Intercellular vacuoles are present. Cell and nuclear membranes are indistinct in places. *i*, intercellular vacuole; s, acidophil granules.

dispersed granules and intracellular vacuoles. Sometimes, however, the acidophil substance in the outer regions of the peripheral cells may itself be disposed in lines perpendicular to the surface of the gland, thus contributing to the general aspect of outwardly radiating lines.

While the secretory granules are appearing, the nuclei of the normal secretory cells often become swollen and vacuolated, and the nuclear membranes become more delicate, until in some places no boundary between nucleus and cytoplasm can be distinguished (Figs. 5 and 8). However, acidophil granules were never found in the nucleus. When the nucleus acquires the swollen aspect, chromatin-like granules are usually found in the cytoplasm near it (Fig. 5). Mitoses were never found in secretory cells that had enlarged sufficiently to be distinguished from undifferentiated cells.

The giant secretory cells (Figs. 1, 2 and 8) are polyploid cells which arise from nuclear mitoses not followed by cytoplasmic division. The nucleus is two to four times larger than the nuclei of normal secretory cells. It is polymorphic, never spherical; its chromatin stains deeply, and it usually contains two to four nucleoli. When the nucleus is undergoing polyploid mitosis, the quantity of cytoplasm is about the same as that of normal diploid cells at rest, but thereafter it expands until the cytoplasmic volume is proportional to the chromatin content. As the cytoplasm becomes more abundant, the cell bodies tend to acquire an increasingly irregular shape. However, in every respect except size the giant secretory cells are similar to the normal ones. During the secretory phase acidophil granules appear in the same concentration as in normal secretory cells. Intracellular vacuoles develop. The nuclear membrane becomes gradually less distinct, and chromatin-like granules are frequently found in the cytoplasm. Most of the giant cells are located in the central portion of the gland, and during stages of intense secretory activity the gland, in sections through its center, may tend to have a stratified appearance (Fig. 8) with a central region containing giant cells and areas of dense cytoplasm, a more peripherally located region rich in intra- and intercellular vacuoles and an outer region in which the nuclei of secretory cells and undifferentiated cells are often arranged side by side to form a border. Due to cellular enlargement and the formation and growth of vacuoles, the gland expands during secretory activity.

Polyploid cells were found in which the chromosome number, counted in the equatorial plate, was between 90 and 100. The cells resulting from such mitoses must be octoploid since the normal diploid number for *Melanoplus differentialis* is 24 (King and Slifer, 1934). This large number of chromosomes was not very often encountered, but tetraploid cells are common in the corpora allata. In a few instances the number of polyploid mitoses in the corpora allata of recently molted nymphs was counted and compared with the number of giant cells in glands of the same nymphal stage which had passed the period of strong mitotic activity. This counting was most conveniently done on sixth instar corpora allata since here it is easy to know when the peaks of cell division and secretory activity are reached. A total of 5 polyploid mitoses, one of them octoploid, was counted in a series of 5 corpora allata from females examined 0 to 4 days after molting; therefore one in each gland. After the fourth day no more polyploid mitoses were found. From 3 to 8, or an average of 3.6 giant cells per gland, were found in females that had spent 5 to 7 days in the sixth stage.

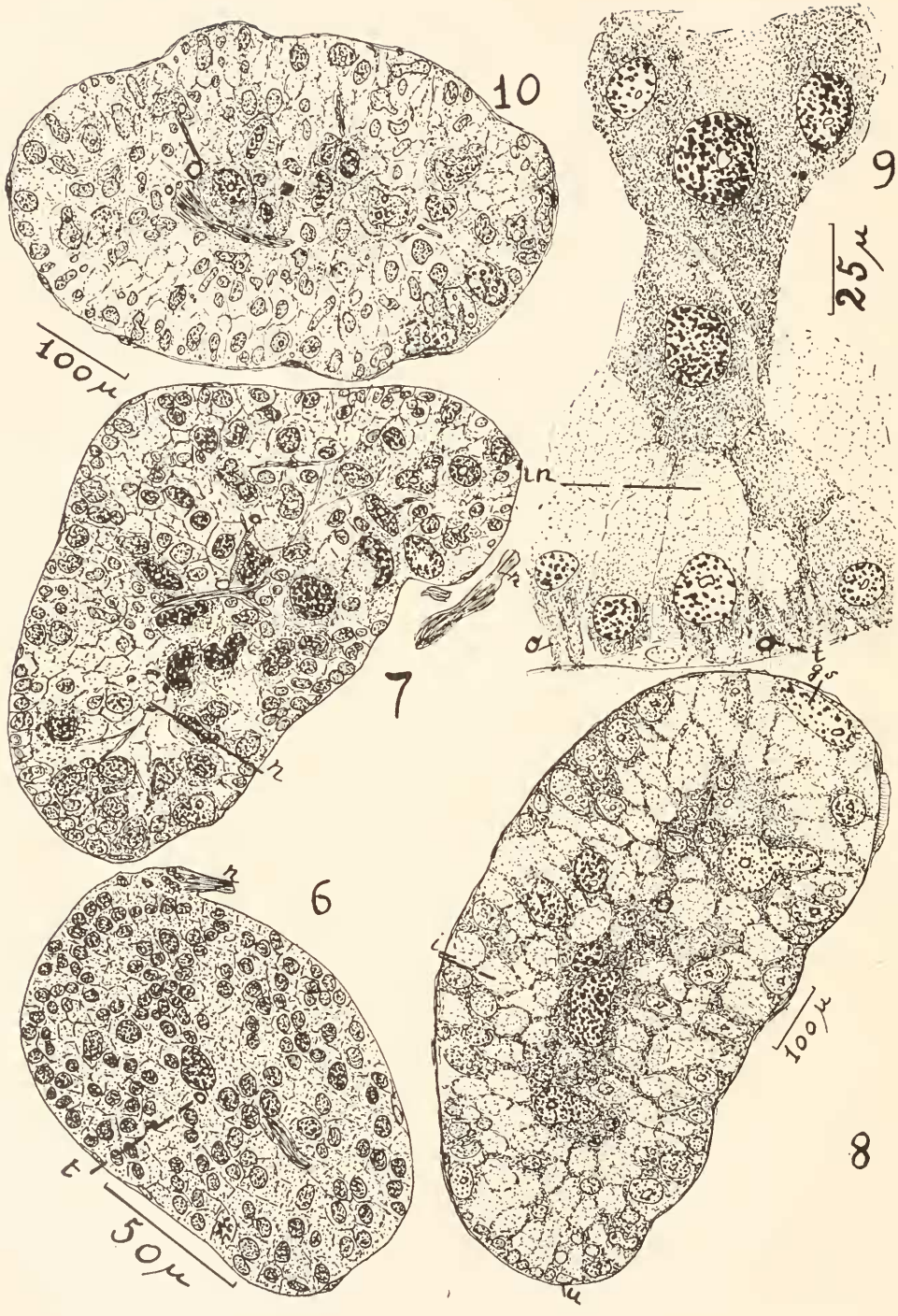
Polyploid mitoses were found only during the early part of each of the nymphal stages. In adults also, polyploid mitoses were most abundant during the days immediately following molting, but occasionally they were found later. The number of giant cells is progressively greater in each stage. Male corpora allata contain about the same number of giant cells as do the corpora allata of females of the same stage, but they are more prominent in the males because male corpora allata are slightly smaller and contain fewer normal secretory cells. The smaller size of the male corpora allata is presumably related to the fact that the males have a smaller total body size than do the females.

Fifth instar corpora allata. The corpora allata of newly molted fifth instar nymphs (Fig. 1) are subspherical, their major and minor axes measuring about 150 and 100 micra, respectively, in females, and about 120 and 100 micra in males. They contain both normal secretory and polyploid cells, as well as undifferentiated cells. The polyploid cells are readily distinguishable by their large nuclei. During the first two or three days of the fifth stage, in grasshoppers having a normal fifth stage intermolt period of seven to eight days, both diploid and polyploid mitoses occur. Mitosis continues up to the fourth to fifth days but less frequently. Acidophil granules are present in the secretory cells from the day the nymph enters the fifth stage until the next molt occurs. They are most abundant from the fourth to sixth days, the cytoplasm of the secretory cells at this time having an intensely acidophil reaction due to the closely crowded granules. Intra- and intercellular vacuoles are also abundant during the fourth to sixth days. The latter tend to increase in size after their first appearance, and the gland shows evidence of active release of secretory material from the fourth day on. By the seventh or eighth day (Fig. 2) both granules and vacuoles become less numerous, and the cells begin to decrease in size.

Sixth instar corpora allata. Just after the grasshoppers enter the sixth stage (Fig. 3) the corpora allata are subspherical, the length of their major and minor axes being around 180 and 130 micra in females and around 150 and 120 micra in males. At this stage all of the cells are similar in appearance, resembling undifferentiated cells. Both normal and polyploid mitoses are present, and immediately after molting the rate of mitotic activity is greatly accelerated, so that during the first three days, in grasshoppers having a sixth stage intermolt period of twelve to fifteen days, one to four and even five mitoses may be found in each section of eight micra. Mitoses are somewhat more numerous in females than in males, as might be expected since the male corpora allata are smaller and contain fewer cells.

On the second and third days after molting, although numerous mitoses are still present in the gland, the cytoplasm of many of the cells begins to increase in volume, and on the third and fourth days normal and giant cells are definitely distinguishable from undifferentiated cells. The cytoplasmic phenomena are the same in sixth stage corpora allata as described for fifth instar nymphs, but the period of secretory activity is briefer and the steps of the secretory process are more clearly defined. During the first two or three days of the sixth stage acidophil granules are absent from the gland. They begin to appear on the third or fourth day (Fig. 4). By the fifth day intra- and intercellular vacuoles are numerous and the "lines of flow" described earlier may be seen directed toward the periphery of the gland. The appearance of the gland at this time suggests that secretion is being released into the body cavity. However, from the seventh or eighth day

PLATE II



until the end of the sixth stage the gland shows no signs of active production of secretion, and mitoses are infrequent. During this stage the giant cells shrink in both nuclear and cytoplasmic volume until it becomes impossible to tell them from normal diploid cells (Fig. 6).

Adult female corpora allata. In newly molted adult females the corpora allata are somewhat flattened bodies, usually roughly circular in outline. Their major and minor axes measure about 400 and 200 micra, respectively. At this time all of the cells are small and similar in appearance, as they were at the close of the sixth stage. However, immediately after molting, in grasshoppers developing at a rate such that oviducal secretion can be expected to appear on the seventh or eighth day of the adult stage, the corpora allata begin to enlarge rapidly, due to cell proliferation, to cytoplasmic expansion and to increase in nuclear volume (Fig. 7). During the first four or five days of the adult stage both normal and polyploid mitoses are present. They are found frequently until the first group of oocytes reach 0.6 mm. in length, but decrease in number during the period when the oocytes grow from 0.6 to 0.75 mm. By the time the production of oviducal secretion and yolk begins mitoses are rare, although they may occasionally be seen throughout the remainder of the adult stage, presumably to replace worn out cells.

Acidophil granules are lacking at the time the female enters the adult stage, but they may be present in some of the secretory cells by the second day after molting if development is rapid. Intra- and intercellular vacuoles may be present by the third or fourth day, with the gland having the appearance that release of secretion to the body cavity has begun by the fourth day. By the fifth or sixth day the gland may have the appearance of intense secretory activity, with the nuclei and cytoplasm greatly expanded, intra- and intercellular vacuoles large and the "lines of flow" strongly evident. The adult corpora allata usually show considerably greater expansion during active phases than is usual for the nymphal corpora allata.

After the secretory phase of the adult corpora allata has begun, it continues at a high level (Fig. 9) throughout the sexually active life of the female. The cytological and histological aspects remain the same and show no variation with blood color changes or with the egg development cycles. When the adult female

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Explanation of Figures

6. Corpus allatum of sixth instar female nymph killed 8 days after molting. Almost all of the cells look alike. Acidophil granules and vacuoles are absent. *t*, trachea.

7. Corpus allatum of adult female killed 2 days after molting. The four types of cells are readily recognizable. Acidophil granules are beginning to appear. *n*, nerve.

8. Corpus allatum of adult male killed 15 days after molting. The gland is at the height of secretory activity. Acidophil granules and vacuoles are present. The gland has a stratified appearance due to the distribution of the granule-filled cytoplasm and the vacuoles. *gs*, giant secretory cell with vacuolated nucleus, nuclear membrane indistinct in places; *i*, intercellular vacuoles; *u*, undifferentiated cells.

9. Portion of the outer region of a corpus allatum of an adult female killed 26 days after molting. The flow of secretory material toward the outside is shown at the periphery of the gland. *in*, intracellular vacuole; *o*, "lines of flow"; *t*, trachea.

10. Corpus allatum of an adult female killed 60 days after the last molt. The first signs of senescence are present. Acidophil granules are becoming scarce. Connective tissue is becoming more prominent both in the outer membrane and within the gland.

becomes old the amount of secretion in the corpora allata gradually diminishes, the outer membrane increases in thickness and the cytoplasm is stained with more difficulty (Fig. 10). Mitoses are absent and the gland decreases somewhat in size.

Adult male corpora allata. Except for their smaller size, the fewer normal secretory cells and the prominence of the giant cells, the corpora allata of adult males (Fig. 8) are histologically very similar to those of adult females developing at the same rate. The corpora allata of newly molted males are usually roughly circular in outline and somewhat flattened. The cells are small, resemble one another, and are devoid of acidophil granules. During the early part of the adult stage there is a period of cell proliferation and nuclear and cytoplasmic expansion. Both normal and polyploid mitoses occur frequently during the first three or four days after molting, then gradually decrease in number. As the cells expand, acidophil granules and then intra- and intercellular vacuoles appear. The corpora allata attain full secretory activity at about the same time in males as in females, and they show the same great expansion of the nuclei and cytoplasm during the active phase.

Discussion

Evidence from experimental studies supports the concept that in hemimetabolous insects (*Rhodnius*, Wigglesworth, 1934, 1936, 1940; *Dixippus*, Pflugfelder, 1937, 1937a, 1939; *Leucophaea*, Scharrer, 1946), as well as in holometabolous insects (*Bombyx*, Bounhiol, 1939), juvenile hormone is released by the corpora allata during the post embryonic stages which precede molts that are accompanied by the development of juvenile (nymphal or larval) characters but is absent or ineffective during the stage which precedes the production of adult characters and metamorphosis. The tissues are evidently stimulated to develop juvenile characters during a "critical period" in the nymphal (or larval) stage when juvenile hormone attains effective concentrations in the blood. That development of the grasshopper *Melanoplus differentialis* conforms to this principle has been suggested by the results of experimental studies (Pfeiffer, 1945) and is now further confirmed by the histological findings of the present investigation. It was seen in this regard that secretory material, presumably juvenile hormone, is elaborated in the corpora allata throughout the greater part of the fifth (next to last) nymphal stage and is most actively released during a period that begins around the middle of the stage and continues until shortly before molting. The "critical period" during which the insect is caused to develop the nymphal characters that appear at the end of the fifth stage may, therefore, be supposed to occur during the latter half of this stage.

On the other hand, the corpora allata of *Melanoplus* also show evidence of secretory activity in the sixth stage, a fact which suggests that juvenile hormone is again being produced. If this is true, it must be concluded that the hormone released in this stage is ineffective in inducing the development of nymphal characters, since metamorphosis occurs at the end of the stage. A reason for such ineffectiveness is suggested by the fact that the entire period of elaboration and release of secretory material in the sixth stage is brief; it does not begin until the second or third day after molting and is completed around the middle of the stage. It would therefore seem probable that the amount of hormone produced is inadequate, or its release may not take place at the proper time to prevent meta-

morphosis. There is also the possibility, suggested by evidence from other insects (*Drosophila,* Bodenstein, 1943; Vogt, 1943; *Leucophaea,* Scharrer, 1946), that the tissues may be less responsive to juvenile hormone in the sixth than in earlier stages, a condition which would further decrease the effectiveness of such amounts of hormone as are produced. In regard to these possibilities, Pfeiffer (1945) has shown that metamorphosis can be delayed in *Melanoplus,* and nymphal characters caused to appear at the end of the sixth stage, by supplying additional amounts of corpus allatum hormone.

Wigglesworth (1934) has also described histological evidence of secretory activity in the corpus allatum of *Rhodnius* during the last (fifth) nymphal stage but was unable by experimental means to detect the presence of juvenile hormone in the blood. On the basis of recent experiments (1947) he has postulated that the corpora allata of old fifth stage nymphs or young adults of *Rhodnius* cause removal of juvenile hormone from the blood. No evidence of a similar function has been observed in the histology of the corpora allata of *Melanoplus.* However, that hormone from the corpora allata may fall to low concentrations in the blood or disappear at the end of the last nymphal stage in this insect, and that it does not regain high levels until about the time the influence of the corpora allata on adult functions (Pfeiffer, 1939, 1945a) is manifest, is suggested by the histological evidence. The corpora allata, it was seen, show no evidence of secretory activity during the last six or seven days of the sixth stage, or on the day of entering the adult stage, but achieve the histological appearance of strong secretory activity shortly before the production of oviducal secretion and yolk begins. Pfeiffer (1945a) has shown in this connection that metabolic conditions in females of *Melanoplus* during the early part of the adult stage are like those continuously in effect when the corpora allata have been removed, but change when there is sufficient hormone in the blood to induce the production of oviducal secretion and yolk.

It is of interest that after the corpora allata have attained high levels of secretory activity in adult females, they continue functioning at such levels, showing no histological evidence of cyclic changes related to the egg production cycles. Joly (1945) likewise found no histological evidence of intermittent activity of the corpora allata of *Dytiscus* but reasoned from experimental evidence that the corpora allata function cyclically in adults of this insect. Also of interest is the fact that the corpora allata of adult males of *Melanoplus* show evidence of as intense secretory activity per unit of glandular tissue as do those of adult females, suggesting that the concentration of hormone in the blood may be maintained at similar levels in both sexes. The smaller size of the corpora allata in the males seems to be related to the smaller total body size of the males rather than to a functional sexual dimorphism. Considering that the corpora allata have a number of functions related to reproduction in adult females of *Melanoplus* (Pfeiffer, 1939, 1945a), but have so far not been found to have comparable importance in males, it could have been expected that they would show a lower level of activity in the males. The histological evidence would therefore seem to support the idea, which has been gaining increasing favor (Pflugfelder, 1938a; Day, 1943; Pfeiffer, 1945a), that the various functions of the corpora allata (see reviews by Hanström, 1939; Scharrer, 1941, 1948; Bodenstein, 1942; Joly, 1945a; Mendes, 1947) are accomplished, in part at least, through effects on metabolic processes rather than through specific actions on the different tissues involved.

In regard to whether the corpora allata secrete different hormones to perform different functions, it can only be said from the present observations that no evidence could be detected histologically that more than one hormone is produced either simultaneously or in the different stages. On the other hand, the fact that the corpora allata of adult grasshoppers may assume an appearance of more intense secretory activity than is seen in nymphs presents the possibility that the concentration of hormone from the corpora allata may reach higher levels in the blood of the adults and that this may have some significance in connection with the different functions of the corpora allata in nymphs and adults. It has been suggested (Bodenstein, 1943; Scharrer, 1946) that differences in hormone concentration and responsiveness of the tissues may play an important part in this connection.

The corpora allata have been described, both topographically and histologically for a large number of insects, the most comprehensive study in this regard having been made by Nabert (1913). References to the many recent descriptions may be found in papers by Hanström (1942); Poulson (1945) and Mendes (1947). The histological characteristics of the corpora allata of *Melanoplus* agree in a number of respects with those described for other insects. Among these may be mentioned: the acidophil reaction of the secretory granules; irregular shape of the cells and nuclei under certain conditions; abundance of chromatin; and the presence of large nucleoli. The occurrence of fine fibrils or striations which may constitute the same phenomenon as that described as "lines of flow" in the present study has been reported to occur in the corpora allata of termites (Pflugfelder, 1938) and in *Gryllotalpa* (De Lerma, 1932). Vacuoles are usually mentioned as a characteristic of active corpora allata. However, Wigglesworth (1934) did not find vacuoles in the corpus allatum of *Rhodnius* when the gland was active, but instead saw them between the cells when the latter had shrunk during a period of inactivity. In *Melanoplus*, it was seen, intercellular vacuoles were present at the height of secretory activity.

It is a general characteristic of the corpora allata that they expand during secretory activity and shrink during inactivity. The expansion may be due simply to enlargement of the cells or, as has been seen in *Melanoplus*, to both cellular expansion and the presence of large vacuoles. There may also be differences in the fundamental size of the gland which are related to the body size of the insect and are based on differences in the number of cells in the gland. In *Melanoplus*, it was seen that the corpora allata are larger and contain more cells in the females than in the males, which in all stages are, on the average, smaller individuals than are the females. The corpora allata also becomes progressively larger in each developmental stage. In agreement with other hemimetabolous insects (*Rhodnius*, Wigglesworth, 1934; *Dixippus*, Pflugfelder, 1937), the latter growth takes place during a period of cell proliferation which occurs at the beginning of the developmental stages. *Rhodnius* differs from *Melanoplus* and *Dixippus*, however, in that there is no proliferative phase at the beginning of the adult stage (Wigglesworth, 1934).

The cells of the corpora allata have generally been described as approximately alike, the principal variations being those shown by all of the cells of the gland in relation to activity and inactivity. However, in the corpora allata of fourth instar nymphs of *Rhodnius*, Wigglesworth (1934) distinguishes between centrally located cells which become swollen and acidophil during the critical period, and periph-

erally located, dividing and growing cells which do not become acidophil. These differences suggest a distinction between undifferentiated cells and secretory cells similar to that seen in *Melanoplus*. De Lerma (1932) found giant cells in *Gryllotalpa* which may be comparable to those observed in *Melanoplus* and which he describes as arising from the fusion of several cells. In *Melanoplus*, on the other hand, it was seen that the giant cells are the result of polyploid mitoses. Since these mitoses occur during the early part of each developmental stage, when mitotic activity is most intense and when the gland is entering an active secretory phase, it seems unlikely that they represent a degenerative condition. Instead, the fact that they have a large volume of cytoplasm in which is elaborated secretory material that cannot be distinguished histologically from that produced by the normal secretory cells suggests that they may be a means of increasing the secretory effectiveness of the gland. It is of interest that the giant cells make up a greater proportion of the gland in males then in females.

It seems probable that the polyploid chromosome content of the giant cells is the result of two or more mitoses of the nuclei of undifferentiated cells taking place in rapid succession. This hypothesis is supported by the fact that the volume of cytoplasm in these cells remains small until division of the nucleus is completed, suggesting that the nuclear divisions are not separated by periods of cytoplasmic expansion. The normal secretory cells, on the other hand, evidently arise from normal diploid division of the undifferentiated cells. This process occurs principally during the proliferative period in each developmental stage, but may occur at other times. Since undifferentiated cells are present in the gland at all times they constitute a reserve from which additional cells may be produced during the time the gland is in the secretory phase. They are also carried over from one stage to the next and are probably, in part at least, the cells in which the new wave of mitotic activity originates. It is possible, however, that the normal secretory cells may also undergo mitosis when they are reduced in size during inactive periods.

Summary

1. The corpora allata of the grasshopper *Melanoplus differentialis* were studied histologically to determine whether these glands show changes related to their functions in the control of nymphal development and the production of ripe eggs and oviducal secretion.

2. Four classes of cells were recognized in the corpora allata of this insect: a, connective tissue and tracheal cells; b, undifferentiated cells; c, normal secretory cells; and d, giant secretory or polyploid cells.

3. The undifferentiated cells are small cells which divide mitotically and do not contain secretory granules or vacuoles. They show the greatest mitotic activity at the beginning of each developmental stage but occasionally divide at other times.

4. The glandular cells, both normal and polyploid, show characteristic changes related to secretory activity and inactivity which are fundamentally the same in nymphs and adults and in both sexes. During inactive phases they are reduced in size and resemble undifferentiated cells. During active phases they increase in cytoplasmic and nuclear volume, the cytoplasm becomes filled with strongly

acidophil granules, then intracellular and finally intercellular vacuoles appear. At the height of secretory activity the appearance of the gland suggests that materials are flowing from central regions of the gland to the periphery.

5. In the fifth nymphal stage, at the end of which nymphal characters are produced, secretory material is elaborated throughout the intermolt period; active release of secretion occurs during the latter half of the stage. In the sixth stage, which terminates with the production of adult characters and metamorphosis, the glands show histological evidence of activity only during a brief period which begins on the second or third day after molting and ends around the middle of the stage.

5. The corpora allata are inactive on the day the grasshopper enters the adult stage, but the histological changes which lead to the production and release of secretory material begin immediately thereafter. Full secretory activity is achieved in the corpora allata of adult females shortly before the onset of the production of oviducal secretion and yolk. It is reached at an equivalent time in adult males.

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